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PTO/SB/05 (12/97) (modified)
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Patent and Trademark Office U S DEPARTMENT OF COMMERCE

UTILITY PATENT APPLICATION TRANSMITTAL

(Only for new nonprovisional applications under 37 CFR 1 53(b))

Attorney Docket No.	312762001700	Total Pages	20
First Named Inventor or Application Identifier			
Ming ZHAO			

JC675 U.S. PTO 09/427699 10/27/99

JC670 U.S. PTO 10/27/99

CERTIFICATE OF HAND DELIVERY

I hereby certify that this correspondence is being hand filed with the United States Patent and Trademark Office in Washington, D.C on October 27, 1999.

Sherri N. Shipe

APPLICATION ELEMENTS

See MPEP chapter 600 concerning utility patent application contents

ADDRESS TO: **Assistant Commissioner for Patents, Box Patent Application, Washington, DC 20231**

1. [X] Fee Transmittal Form *(Submit an original, and a duplicate for fee processing)*
2. [X] Specification [Total Pages 22] *(preferred arrangement set forth below)*
   - Descriptive title of the Invention
   - Cross References to Related Applications
   - Statement Regarding Fed sponsored R & D
   - Reference to Microfiche Appendix
   - Background of the Invention
   - Brief Summary of the Invention
   - Brief Description of the Drawings *(if filed)*
   - Detailed Description
   - Claim(s)
   - Abstract of the Disclosure
3. [ ] Drawing(s) *(35 USC 113)* [Total Sheets [ ]]
4. [X] Oath or Declaration (unexecuted) Total Pages [3]
   a. [ ] Newly executed (original or copy)
   b. [ ] Copy from a prior application (37 CFR 1.63(d)) *(for continuation/divisional with Box 17 completed)* ***[Note Box 5 below]***
      i. [ ] DELETION OF INVENTOR(S) Signed statement attached deleting inventor(s) named in the prior application, see 37 CFR 1 63(d)(2) and 1 33(b)
5. [X] Incorporation By Reference *(useable if Box 4b is checked)* The entire disclosure of the prior application, from which a copy of the oath or declaration is supplied under Box 4b, is considered as being part of the disclosure of the accompanying application and is hereby incorporated by reference therein
6. [ ] Microfiche Computer Program *(Appendix)*
7. Nucleotide and/or Amino Acid Sequence Submission *(if applicable, all necessary)*
   a. [ ] Computer Readable Copy
   b. [ ] Paper Copy (identical to computer copy)
   c. [ ] Statement verifying identity of above copies

ACCOMPANYING APPLICATION PARTS

8. [ ] Assignment Papers (cover sheet & document(s))
9. [ ] 37 CFR 3.73(b) Statement [ ] Power of Attorney *(when there is an assignee)*
10. [ ] English Translation Document *(if applicable)*
11. [ ] Information Disclosure Statement (IDS)/PTO-1449 [ ] Copies of IDS Citations
12. [ ] Preliminary Amendment
13. [X] Return Receipt Postcard (MPEP 503) *(Should be specifically itemized)*
14. [X] Small Entity Statement(s) [X] Statement filed in prior application, Status still proper and desired
15. [ ] Certified Copy of Priority Document(s) *(if foreign priority is claimed)*
16. [ ] .....

17. If a CONTINUING APPLICATION, *check appropriate box and supply the requisite information:*

[ ] Continuation [ ] Divisional [X] Continuation-in-part (CIP) of prior application No: 08/859,051

18. CORRESPONDENCE ADDRESS

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1

Due PTO did not receive the following listed items: 22 pages of specification
We did received 12 pages

☒ If a paper is untimely filed in the above-referenced application by applicant or his/her representative, the Assistant Commissioner is hereby petitioned under 37 C.F.R. § 1.136(a) for the minimum extension of time required to make said paper timely. In the event a petition for extension of time is made under the provisions of this paragraph, the Assistant Commissioner is hereby requested to charge any fee required under 37 C.F.R. § 1.17(a)-(d) to **Deposit Account No. 03-1952.** However, the Assistant Commissioner is **NOT** authorized to charge the cost of the issue fee to the Deposit Account.

The filing fee has been calculated as follows:

FOR	NUMBER FILED	NUMBER EXTRA	RATE	CALCULATIONS
TOTAL CLAIMS	12 - 20 =	0	x $18.00	$0
INDEPENDENT CLAIMS	2 - 3 =	0	x $78.00	$0
MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+ $260.00	$0
			BASIC FEE	$380.00
		TOTAL OF ABOVE CALCULATIONS =		$0
Reduction by 1/2 for filing by small entity (Note 37 C.F.R. §§ 1.9, 1.27, 1.28). If applicable, verified statement must be attached.				$0
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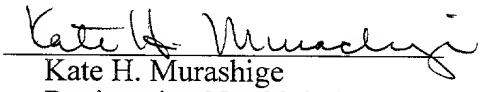
☒ A check in the amount of $380.00 is attached.

Applicant(s) hereby petitions for any required relief including extensions of time and authorizes the Assistant Commissioner to charge the cost of such petitions and/or other fees or to credit any overpayment to **Deposit Account No. 03-1952** referencing docket no. 312762001800. A duplicate copy of this transmittal is enclosed, for that purpose.

Dated: October 27, 1999

Respectfully submitted,

By: 

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Applicant/Patentee: Ming ZHAO et al. Docket No.: 312763001800
Serial No./Patent No.: 60/105,831
Filed on/Issued : 27 October 1998
For: CONSTRUCTION OF p21-GFP FUSION GENE AND SELECTIVE DELIVERY TO MOUSE HAIR FOLLICLES

VERIFIED STATEMENT CLAIMING SMALL ENTITY STATUS
37 C.F.R. §§ 1.9(f) AND 1.27(c) – SMALL BUSINESS CONCERN

I hereby declare that I am

☐ the owner of the small business concern identified below:
☒ an official of the small business concern empowered to act on behalf of the concern identified below:

NAME OF CONCERN: AntiCancer, Inc.

ADDRESS OF CONCERN: 7917 Ostrow Street, San Diego, CA 92111

I hereby declare that the above identified small business concern qualifies as a small business concern as defined in 13 C.F.R. § 121.12, and reproduced in 37 C.F.R. § 1.9(d), for purposes of paying reduced fees to the United States Patent and Trademark Office, in that the number of employees of the concern, including those of its affiliates, does not exceed 500 persons. For purposes of this statement, (1) the number of employees of the business concern is the average over the previous fiscal year of the concern of the persons employed on a full-time, part-time or temporary basis during each of the pay periods of the fiscal year, and (2) concerns are affiliates of each other when either, directly or indirectly, one concern controls or has the power to control the other, or a third party or parties controls or has the power to control both.

I hereby declare that rights under contract or law have been conveyed to and remain with the small business concern identified above with regard to the invention, entitled METHIONINASE GENE THERAPY FOR TUMOR TREATMENT by inventors Mingxu XU and Yuying TAN described in

☐ the specification filed herewith with title as listed above.
☒ the application identified above.
☐ the patent identified above.

If the rights held by the above identified business concern are not exclusive, each individual, concern or organization having rights in the invention must file separate verified statements averring to their status as small entities, and no rights to the invention are held by any person, other than the inventor, who would not qualify as an independent inventor under 37 C.F.R. § 1.9(c) if that person made the invention, or by any concern which would not qualify as a small business concern under 37 C.F.R. § 1.9(d), or a nonprofit organization under 37 C.F.R. § 1.9(e).

Each person, concern or organization having any rights in the invention is listed below:

☒ no such person, concern, or organization exists.
☐ each such person, concern or organization is listed below.

NAME	ADDRESS	TYPE
		☐ Individual ☐ Small Business Concern ☐ Nonprofit Organization

Separate verified statements are required from each named person, concern or organization having rights to the invention averring to their status as small entities. (37 C.F.R. § 1.27)

I acknowledge the duty to file, in this application or patent, notification or any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 C.F.R. § 1.28(b))

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

NAME OF PERSON SIGNING: Rey Magana
TITLE OF PERSON IF OTHER THAN OWNER: General Manager
ADDRESS OF PERSON SIGNING: AntiCancer, Inc.
7917 Ostrow Street
San Diego, CA 92111

SIGNATURE: _____ DATE: February 2, 1999

PTO/SB/10 (10-92)
dc-146678

TREATMENT OF ALOPECIA

This application is a continuation-in-part of U.S. Serial No. 08/859,051, filed 20 May 1997 which is a divisional of U.S. Serial No. 08/858,469, filed 20 May 1997 and now U.S. Patent No. 5,914,126 which is a divisional application of U.S. Serial No. 08/486,520, filed 7 June 1995 and now U.S. Patent No. 5,753,263. This application also claims priority under 35 U.S.C. § 119 from provisional application 60/105,831 filed 27 October 1998. The contents of these applications are incorporated herein by reference.

Technical Field

This invention relates to methods for specifically delivering cell cycle inhibitors to hair follicles to inhibit alopecia. In particular, the invention relates to specifically delivering the cell-cycle inhibitor, p21 to hair follicles.

Background Art

Many chemotherapeutic agents, for example, antimetabolites (methotrexate, 5-fluoouracil, cytarabine), alkylating agents (cyclophosphamide, mechlorethamine, dacarbazine, ifosfamide), antineoplatic antibiotics (bleomycin, actinomycin D, daunomycin, doxorubicin, mitoxantrone), the vinca alkaloids (vincistine, vinblastine) and taxanes (Taxol, Taxotere), produce an anagen effluvium to induce alopecia by killing the active proliferating cells of the hair matrix. Scalp hair is particularly sensitive since 85% of scalp hair is in anagen phase. Chemotherapy-induced alopecia (CIA) is thus a major problem in clinical oncology, which can be a major obstacle for patients to accept chemotherapy. It is particularly devastating to women as alopecia-inducing agents such as the taxanes are becoming more frequently used in breast and ovarian cancers.

Surgical transplantation of small, discrete, skin areas having viable follicles to areas having inactive follicles is expensive, labor-intensive and relatively short-lasting. Also, as described by R. F. Oliver *et al.* in U.S. Pat. No. 4,919,664, follicular dermal cells can be inserted into a skin incision, resulting in hair growth along the incision. However,

this is a complex technique that does nothing to stimulate existing follicles. Treatment of the hair and skin with various creams or lotions with biologically active ingredients to improve hair growth has generally low efficiency. Attempts to follow this approach have been ineffective, possibly because of the inability of stimulators to penetrate the cellular membrane of hair follicle cells and to enter into the cells where their action is needed.

Liposomes, which are artificial phospholipid vesicles, have been successfully used for delivery of different low-molecular-weight water-soluble and oil-soluble compounds into different cells. See, for example, G. Gregoriadis, *Trends in Biotechnology* (1985) 3:235-241 and K. H. Schmidt, ed., Liposomes as Drug Carriers, Stuttgart:George Thieme Verlag (1986). The applications and issued patents from which priority is claimed describe the use of liposomes to target hair follicles specifically.

No pharmacological agent inhibits CIA in a reliable, cost-efficient, unharmful and long-lasting manner. The treatment of human hair loss, especially CIA is important and beneficial for cancer patients and other persons generally. Thus, new agents and treatments for the prevention of CIA are needed.

It has been verified, as exemplified below, that chemotherapy-induced alopecia can be almost completely prevented in skin histoculture by liposome targeting of the gene encoding the cyclin-dependent-kinase inhibitor p21 to the hair follicle.

Summary of the Invention

Generally, the present invention is directed to methods to inhibit chemotherapy-induced alopecia by selective delivery of expression systems for cell cycle inhibitors to the hair follicles.

In another aspect, the invention is directed to a method to monitor the expression of p21 in cells which method comprises assessing the fluorescence of the cells that have been treated with an expression system for a fusion protein, which comprises an amino acid sequence conferring p21 activity linked to an amino acid sequence which effects

emission of fluorescent light. The fluorescence may be observed *in vitro* or *in vivo*, even in the live subject.

Detailed Description of the Invention

Cyclin-dependent kinase inhibitor (CDIs) proteins are important because they regulate CDKs at specific points in the cell cycle and can therefore negatively control progression of the cell cycle. See Hirama *et al. Blood* (1995) 86:841-854; Polyak *et al. Gene Dev* (1994) 8:9; Toyoshima *et al. Cell* (1994) 78:67; Serrano *et al. Nature* (1993) 366:704; Serrano *et al. Science* (1995) 267:249. The mammalian CDIs include p21, p20, p27, p28, p16, p15 and p18. The p21 protein is a potent inhibitor of all cyclin/CDK complexes tested, including cyclin/CDK2 and cyclin D/CDK4. The p21 protein can inhibit DNA replication in the absence of cyclin/CDK by binding to PCNA and inhibiting its ability to activate DNA polymerase δ and thus p21 can stop DNA synthesis and inhibit cells from entering S-phase. Waga *et al. Nature* (1994) 369:574. Therapeutic application of p21 puts the hair-follicle into a resting state (telogen) to protect against toxic events such as chemotherapy-induced alopecia (CIA).

The hair follicle is a complex mini-organ driven by a biological clock in a rhythmic, cyclic fashion from stages of resting (telogen) to growth (anagen) and via a short regression phase (catagen) back to the telogen phase. See Chase, H.B. *Physiol Rev* (1954) 34:113-26; Orfanos, C.E., Happle, R. (eds.) Hair Growth and Hair Diseases, Springer, Berlin (1990); Rook, A., Dawber, R. (eds.) Diseases of the Hair and Scalp, Blackwell, Oxford (1992). Cytotoxic agents damage cycling hair matrix cells to cause "anagen effluvium" and eventually induce alopecia. See Hood *et al.* Cancer Medicine, 4th ed. Williams & Wilkins. 1:3141 (1997).

An *in vitro* model for studying the anagen phase of the murine hair cycle for almost the entire duration has been developed. In this model, catagen can be induced by chemotherapeutic drugs. This *in vitro* system is based on the collagen-sponge-gel-matrix supported histoculture technology adapted for hair-producing skin culture. See Li *et al.*

Proc Natl Acad Sci USA (1992) 88:1908-12; Li *et al. In vitro Cell Dev Biol* (1992) 28A:479-481, 695-698, 679-681; Li *et al. In vitro Cell Dev Biol* (1993) 29A:192-194, 258-260, 449-450; Li *et al. In vitro Cell Dev Biol* (1994) 30A:135-138. The skin histoculture technology provides an opportunity to observe, characterize, analyze and manipulate the development of anagen (growth) and catagen (breakdown) in mature hair follicles *in vitro* in full-thickness skin. The skin histoculture system may also be used to induce CIA with actual hair loss observed *in vitro*.

Recently, two animal models have been used for studying CIA. It has been demonstrated that the new-born rat is a model for CIA with doxorubicin and cyclophosphamide/cytosine arabinoside. See Jimenez *et al. Cancer Invest* (1992) 10:271-276. The doxorubicin-induced alopecia in new-born rats was prevented by 1,25-dihydroxyvitamin D_3 and the cyclophosphamide-induced alopecia was prevented by ImuVert/N-acetylcysteine. Also reported is a murine model for inducing and manipulating hair follicle regression (catagen) and CIA. See Paus *et al. J Invest Derm* (1994) 103:143-147; Paus *et al. Cancer Research* (1996) 56:4438-4443; Paus *et al. Am J Pathol* (1994) 144:719-734. The massive catagen development in anagen C57B1-6 mice can be achieved after topical treatment with dexamethasone once daily. CIA evidenced by dystrophic anagen, catagen and disruption of melanization of hair follicles was established in C57B1-6 mice with a single intraperitoneal injection of cyclophosphamide. See Hoffman, R. M. *J Cell Pharmacol* (1991) 2:189-201. Topical application of immunophilin ligands such as cyclosporin A and FK506 can induce active hair growth in telogen C57B16 mice and also inhibit massive, dexamethasone-induced, premature catagen development. See Maurer *et al. Am J Pathol* (1997) 150:1433-1441.

This invention provides uniquely effective protocols and materials for the treatment of CIA, as well as assay systems for monitoring hair follicle cells and hair growth. The cyclin-dependent kinase (CDK) inhibitors including p21, p16 and p27 may be used to put the hair follicle into a resting, chemo-resistant phase to prevent CIA.

The expression systems employed in the present invention generally comprise a nucleotide sequence encoding a cell-cycle inhibitor such as the p21 protein operably linked to sequences which effect expression of the coding sequence. Vectors for delivery of the nucleotide sequence encoding a cell-cycle inhibitor may also effect the insertion of the nucleotide sequence into the genome of the host, thus employing the endogenous control sequences to effect expression.

If the vector contains an expression system, suitable promoters and enhancers can be used. General constitutive promoters such as SV40 or CMV promoters can be included, along with their enhancer elements, or tissue-specific promoters may be used to enhance specificity. Means to construct suitable vectors for delivery of a gene along with provision for its expression are well known in the art.

In order to effect the modification of cells for the expression of the cell-cycle inhibitor, the expression system or integrating encoding nucleotide sequence must be formulated so as to enter the cell. Integration of the desired nucleotide sequences into viral vectors, such as adenovirus may provide this means of entry. However, retroviral vectors, or other mediators of cellular uptake, such as lipids, or various liposomal type formulations or emulsions are preferred.

It is also part of the invention to employ the protein exhibiting cell-cycle inhibition as a fusion protein to a reporter amino acid sequence, most preferably an amino acid sequence which confers fluorescence on the fusion protein. The use of green fluorescent protein (GFP) to confer fluorescence on a fusion protein is well understood in the art; see, for example, Chalfie, M., *et al. Science* (1994) 263:802-805. The expression system may be targeted to the hair follicle cells of interest by utilizing liposome-mediated delivery as described in U.S. Pat. No. 5,641,508, filed 13 January 1994, and incorporated herein by reference.

The invention contemplates using the expression system to study and treat chemotherapy-induced alopecia (CIA). Similarly, the invention contemplates using the

expression system in *in vitro* and animal models to determine the effects of various substances on CIA.

A therapeutic composition contains the expression system of the present invention. A therapeutically effective amount of the expression system and, if present, other beneficial compounds, is a predetermined amount calculated to achieve the desired effects, i.e., to effectively affect the pigmentation of the skin or hair cells. Thus, an effective amount can be measured by improvements in one or more symptoms associated skin or hair cell growth in the subject.

The dosage can be adjusted by the individual physician in the event of any complication. The compositions are administered in a manner compatible with the dosage formulation, and in a therapeutically effective amount. The quantity to be administered depends on the subject to be treated, capacity of the subject's system to utilize the active ingredient, and degree of therapeutic effect desired. Precise amounts of active ingredient required to be administered depend on the judgement of the practitioner and are peculiar to each individual. However, suitable dosage ranges for systemic application are disclosed herein and depend on the conditions of administration. Suitable regimes for administration are also variable, but are typified by an initial administration followed by repeated doses at one or more hour intervals by a subsequent administration.

The choice of vector to which a DNA segment of the present invention is operatively linked depends directly, as is well known in the art, on the functional properties desired, e.g., protein expression, and the host cell to be transformed, these being limitations inherent in the art of constructing recombinant DNA molecules. However, a vector contemplated by the present invention is at least capable of directing the replication, and preferably also expression, of the beneficial protein structural gene included in DNA segments to which it is operatively linked.

In preferred embodiments, a vector contemplated by the present invention includes a procaryotic replicon, i.e., a DNA sequence having the ability to direct autonomous replication and maintenance of the recombinant DNA molecule

extrachromosomally in a procaryotic host cell, such as a bacterial host cell, transformed therewith. Such replicons are well known in the art. In addition, those embodiments that include a procaryotic replicon also include a gene whose expression confers drug resistance to a bacterial host transformed therewith. Typical bacterial drug resistance genes are those that confer resistance to ampicillin or tetracycline.

Those vectors that include a procaryotic replicon can also include a procaryotic promoter capable of directing the expression (transcription and translation) of the beneficial protein gene in a bacterial host cell, such as E. coli, transformed therewith. A promoter is an expression control element formed by a DNA sequence that permits binding of RNA polymerase and transcription to occur. Promoter sequences compatible with bacterial hosts are typically provided in plasmid vectors containing convenient restriction sites for insertion of a DNA segment of the present invention. Typical of such vector plasmid are pUC8, pUC9, pBR322 and pBR329 available from Biorad Laboratories, (Richmond, Calif.) and pPL and pKK223 available from Pharmacia, Piscataway, N.J.

Expression vectors compatible with eucaryotic cells, preferably those compatible with mammalian cells, and particularly hair follicle cells, can also be used to form the recombinant DNA molecules for use in the present invention. Mammalian cell expression vectors are well known in the art and are available from several commercial sources. Typically, such vectors are provided containing convenient restriction sites for insertion of the desired DNA segment, and provide the signals required for gene expression in a mammalian cell. Typical of such vectors are the pREP series vectors and pEBVhis available from Invitrogen (San Diego, Calif.), the vectors pTDT1 (ATCC #31255), pCP1 (ATCC #37351) and pJ4W (ATCC #37720) available from the American Type Culture Collection (ATCC) and the like mammalian expression vectors.

Particularly preferred are mammalian expression vectors which allow the expression of the gene in a tissue-specific manner, in this case by the action of a regulatory promotor that will limit gene expression to hair follicle cells.

Successful transformation of the target tissue can be confirmed by evaluation of the target tissue for indicia of function exerted by the administered beneficial compound. For example, where the compound is a nucleic acid expressing p21 protein, as described in the Examples, successful transformation can be detected by evaluating the cell cycle phases of the cells in the target tissue.

The following examples are intended to illustrate but not to limit the invention.

Example 1

Cloning of Human p21 Gene

The nucleotide sequence encoding the human p21 gene was amplified by PCR from plasmid MBP-p21, Zhang *et al. Gene* (1994) 3:1750-1758 (1994). Oligomers were designed according to the sequence of the human p21 gene. Xiong *et al.* Nature (1993) 366:701. The upstream primer was

5'-CCG CTC GAG ATG TCA GAA CCG GCT GG-3'.

The downstream primer was

5'-CGC GGA TTC TTA GGG CTT CCT CTT GGA CT-3'.

The PCR primer reaction conditions were as follows: first denaturation at 94°C for 10 min; then 30 cycles of denaturation at 94°C for 30 s; annealing at 50°C for 30 s; and extension at 72°C for 45 s; then a final extension at 72°C for 10 min.

Electrophoretic analysis demonstrated that the amplified products had the predicted size of 500 bp.

Example 2

Construction of pEGFP-p21

The vector pEGFP-C_3 (Clonetech, Palo Alto, CA), encodes a red-shifted variant of wild-type GFP that has been optimized for brighter fluorescence and higher expression in mammalian cells. The multiple cloning site (MCS) in pEGFP-C_3 is between the EGFP

coding sequences and the SV40 polyA. Genes cloned into the MCS will be expressed as fusion to the C-terminus of EGFP if they are in the same reading frame (Neo^R) contained in the vector allows stable transfected eukaryotic cells to be selected using G418.

The 500 bp p21 amplified gene was cloned into the XhoI/BamHI cloning site of the pEGFP-C_3 vector to obtain pEGFP-p21, and correct insertion confirmed by restriction enzyme analysis.

Example 3

Histoculture of Skin and Transfection of Cultured Skin with pEGFP-p21 Vector

Baby balb-c mice (2 weeks) were treated with hair remover. Small pieces of mouse skin (2 x 5 x 2 mm) were cut with a scissors and put onto collagen-containing gels in histoculture in Eagle's minimum essential medium (MEM) supplemented with 10% fetal bovine serum and gentamycin, as described by Li *et al.* PNAS USA (1991) 88:1908-1912. Cultures were maintained at 37°C in a gassed incubator with 5% CO_2. Liposome interaction with the skin was initiated after 24 hours of histoculture.

40 ml of LipoTAXI transfection reagent (Stratagene, San Diego, CA) were added to 20ml (10 μg) of pEGFP-p21 plasmid DNA, then mixed and incubated for 30 min. at room temperature. 400 μl of serum-free MEM was added to the mixture, then transferred to the skin-culture dish with swirling and the mixtures were incubated for 4 hours at 37 °C. The medium was replaced with 2 ml of MEM with 10% serum and incubated for 48 hours at 37° in 5% CO_2.

A Nikon fluorescence microscope, equipped with aGFP cubes was used to observe expression. The EGFP-p21 gene was expressed selectively in hair follicles as visualized by bright GFP fluorescence.

Incorporation by Reference

All publications, patents, and patent applications cited herein are expressly incorporated by reference.

Claims

1. A method to inhibit alopecia which method comprises delivering p21 to hair follicles of a mammal, said delivering comprising the step of applying topically to skin areas of a mammal having a plurality of hair follicles, an effective amount of a nucleotide sequence encoding the p21 protein.

2. The method of claim 1 wherein said nucleotide sequence is operably linked to control sequences to effect its expression.

3. The method of claim 1 wherein said nucleotide sequence is contained in a vector.

4. The method of claim 3 wherein said vector is a viral vector.

5. The method of claim 1 wherein said p21 is contained in a liposomal formulation.

6. The method of claim 1 wherein the alopecia is androgenic or is chemotherapy-induced alopecia.

7. The method of claim 3 wherein said vector is contained in a liposomal formulation.

8. A method to observe the expression of p21 in hair follicle cells which method comprises providing said cells with an expression system for a nucleotide sequence encoding p21 fused to a nucleotide sequence that encodes an amino acid sequence which confers fluorescence.

9. The method of claim 8 wherein the expression system is contained in a plasmid, a retroviral vector or an adenoviral vector.

10. The method of claim 9 wherein the plasmid is pEDFP-p21.

11. The method of claim 9 wherein said cells are contained in a histoculture.

12. A histoculture prepared by the method of claim 11.

Abstract

A composition and method for treating and monitoring alopecia by altering hair follicle cells to produce a cell cycle inhibitor is disclosed.

PATENT
Docket No. 312762001800

DECLARATION FOR UTILITY PATENT APPLICATION

AS A BELOW-NAMED INVENTOR, I HEREBY DECLARE THAT:

My residence, post office address, and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor of the subject matter which is claimed and for which a patent is sought on the invention entitled: TREATMENT OF ALOPECIA, the specification of which is attached hereto unless the following box is checked:

☒ was filed on 27 October 1999 as United States Application Serial No. _____ and was amended on (if applicable).

I HEREBY STATE THAT I HAVE REVIEWED AND UNDERSTAND THE CONTENTS OF THE ABOVE-IDENTIFIED SPECIFICATION, INCLUDING THE CLAIMS, AS AMENDED BY ANY AMENDMENT REFERRED TO ABOVE.

I acknowledge the duty to disclose information which is material to the patentability as defined in 37 C.F.R. § 1.56.

I hereby claim foreign priority benefits under 35 U.S.C. § 119(a)-(d) or § 365(b) of any foreign application(s) for patent or inventor's certificate, or § 365(a) of any PCT International application which designated at least one country other than the United States listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or PCT International application having a filing date before that of the application on which priority is claimed:

Application No.	Country	Date of Filing (day/month/year)	Priority Claimed?
			☐Yes ☐No

I hereby claim benefit under 35 U.S.C. § 119(e) of any United States provisional application(s) listed below:

Application Serial No.	Filing Date
60/105,831	27 October 1998

I hereby claim the benefit under 35 U.S.C. § 120 of any United States application(s), or § 365(c) of any PCT International application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of 35 U.S.C. § 112, I acknowledge the duty to disclose information which is material to patentability as defined in 37 C.F.R. § 1.56 which became available between the filing date of the prior application and the national or PCT International filing date of this application.

Application Serial No.	Filing Date	Status
08/859,051	20 May 1997	☐Patented ☒Pending ☐Abandoned
08/858,469	20 May 1997	☒Patented ☐Pending ☐Abandoned
08/486,520	7 June 1995	☒Patented ☐Pending ☐Abandoned

I hereby appoint the following attorneys and agents to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith:

Sanjay Bagade (Reg No. 42,280)
Richard R. Batt (Reg No. 43,485)
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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these

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